C'
corel

Number 06/784,857, filed October 4, 1985, now abandoned."

Page 27, 2nd paragraph, 3rd line, change "tesetosterone" to "testosterone".

In the Specification, please replace the Tables 20 to 25 as follows:

C2

Table 20.

Tracer	mg/tube:	+ 5 Salicylate	+ 10 Salicylate	+ 0 15 DNP	+ 0.3 DNP	+ 1 0 ANS	+ 2 0 ANS
without SBP	7.9 (A)	13.0 (B)	14.3 (C)	10.5 (D)	13 4 (E)	18 9 (F)	17 7 (G)
with SBP	7 1 (A')	13.1 (B')	14 4 (C')	9 8 (D')	13 5 (E')	16 8 (F')	15 0 (G')

C3

Table 21

A = 1 14	A' · 0.21	r = 0 991
B = 1 00	B' · 0 11	r = 0 997
C = 1 02	C' · 0 31	r = 0 998
D = 1 11	D' · 0 30	r = 0 996

Table 21. (continued)

E = 1.03	E' · 0.52	r = 0.997
F = 1.13	F' · 0.05	r = 0.996
G = 1.19	C' · 0 26	r = 0 997
A' = 2.76	F' · 2.70	r = 0.966
A' = 2.34	G' · 1.58	r = 0 987

C4

Table 22.

Tracer:	6-Hydroxytestosterone-19-histamine-^{125}I	Testosterone-19-histamine-^{125}I
Mean (n = 20)	11.0 (A) A = 1.48 B + 1.24 r = 0.977	17.3 (B)

Table 23.

Sample	Unspiked	Spiked with Albumin (gm/dl)		
		1.0	2.0	3.0
1	4.7	4.4	4.2	3.9
2	16.7	16.4	16.7	15.8
3	37.0	37.0	34 2	34 0
Mean	19 5	19.3	18 4	17 9
Recovery	-	99%	94%	92%

Table 24.

Normal Males		Normal Females		3rd Trimester	
Before	After	Before	After	Before	After
21.24	96%	0.80	105%	4.75	99%
14.33	98%	2.32	104%	9.10	96%
19.91	99%	1.73	104%	3.58	96%
21.03	98%	3.47	104%	4.44	97%
15.01	95%	1.93	105%	3.86	96%

Table 25.

Oleic Acid Added	Patient 1	Patient 2	Patient 3	Mean
0 mmol/l	6.2	3.3	10.0	6.5
2.5	7.3	4.7	11.6	7.9
5.0	11.6	11.1	21.8	14.8
7.5	21.2	16.0	32.3	23.2
10.0	30.3	17.9	47.8	32.0

Please cancel claims 28 to 40 and insert the following.

41. A method for measuring the concentration of free testosterone ligand in a biological fluid in the presence of bound ligand and endogenous binding proteins, without disturbing the

equilibrium between said free ligand and protein-bound ligand, which method comprises

(a) incubating, in the absence of salicylate, 2, 4-dinitrophenol and 8-anilino-1-naphthalenesulfonic acid, a sample of the biological fluid with (i) a ligand analog tracer which, due to its chemical structure, does not bind to some of the endogenous binding proteins but does bind to at least one other endogenous binding protein, (ii) a concentration of a specific ligand binder having an affinity constant and selectivity for the said free ligand such that the equilibrium between free ligand and protein-bound ligand is not disturbed and (iii) a concentration of a specific inhibitor agent that inhibits the binding of the ligand analog tracer to said at least one other endogenous binding protein sufficient to block reaction between the ligand analog tracer and said at least one other endogenous binding protein without displacing ligand from protein-bound ligand;

(b) separating the ligand analog tracer bound to the specific ligand binder from unbound tracer; and

(c) determining the concentration of said free ligand in said biological fluid.

42. A method according to claim 41 wherein, in step (c), the concentration of free ligand in said biological fluid is determined by comparing the bound fraction of ligand analog tracer in said sample to the bound fraction in a given set of free ligand calibrators.

43. A method according to claim 41 wherein the specific ligand binder is an antibody to said free ligand.

44. A method according to claim 41 wherein the specific ligand binder is immobilized on a solid substrate.

45. A method according to claim 41 wherein the solid substrate is polypropylene.

46. A method according to claim 41 wherein the ligand analog tracer is labelled with at least one radioactive atom, an enzyme, fluorophor, light chromophore or chemiluminescent group.

47. A method according to claim 41 wherein the ligand analog tracer is iodinated 6-hydroxytestosterone-19-carboxymethyl ether histamine.

48. A method according to claim 41 wherein the method is carried out at about 37°C and at about pH 7.4.

49. A method according to claim 42 wherein said free ligand calibrators have been prepared by adding different amounts of the ligand to ligand-free human serum, calibrating by equilibrium dialysis and assigning free ligand values.

50. A method according to claim 41 wherein the specific